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Conversion of Quinazoline Modulators from Inhibitors to Activators of β -Glucocerebrosidase

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Abstract

Gaucher's disease is a lysosomal disease caused by mutations in the β -glucocerebrosidase gene (*GBA1*, GCase) that have been also linked to increased risk of Parkinson's disease and Diffuse Lewy Body Dementia. Prior studies have suggested that mutant GCase protein undergoes misfolding and degradation and therefore stabilization of the mutant protein represents an important therapeutic strategy in synucleinopathies. In this work, we present a structure activity relationship (SAR) study of quinazoline compounds that serve as inhibitors of GCase. Unexpectedly, we found that *N*-methylation of these inhibitors transformed them into GCase activators. A systematic SAR study further revealed that replacement of the key oxygen atom in the linker of quinazoline derivative also contributed to the activity switch. PD patient-derived fibroblasts and dopaminergic midbrain neurons were treated with a selected compound (**9q**) that partially stabilized GCase and improved its activity. These results highlight a novel strategy for therapeutic development of non-inhibitory GCase modulators in Parkinson's disease and related synucleinopathies.

Graphical Abstract

Keywords

β -Glucocerebrosidase; Gaucher's disease; Parkinson's disease; Quinazoline; Modulator; SAR

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INTRODUCTION

Gaucher's disease (GD), an autosomal recessive lysosomal storage disorder, is caused by mutations of the *GBA1* gene encoding the lysosomal enzyme β -glucocerebrosidase (GCase). Mutations in *GBA1* were also found as a major risk factor for Parkinson's disease (PD) and dementia with Lewy bodies (DLB)^{1–5}. Most of these mutations, including the prevalent N370S mutation, are still functional, although with low residual GCase activity⁶. Loss of function of GCase leads to accumulation of glucosylceramide (GluCer)^{7,8}, that also promotes the formation and stabilization of α -synuclein oligomers⁹. Enhancement of GCase activity is thought to be a potential therapeutic strategy for GCase-associated synucleinopathies, including PD^{10,11}.

Several different scaffolds of non-iminosugar GCase inhibitors have been reported as GCase pharmacological chaperones (PCs) since 2007^{12–16} (Figure 1). We found that a noninhibitory GCase PC NCGC00188758 (**2**)¹⁷ can enhance GCase activity specifically within the lysosomal compartment, reduce GluCer and hexosylsphingosine substrates, and subsequently enhance the clearance of pathological α -synuclein¹⁸. These findings suggested that reduction of GluCer may provide benefit in PD and strengthen the notion that GCase is a valuable target for the treatment of synucleinopathies.

In our previous work¹⁹, we discovered a series of quinazoline inhibitors with nanomolar potency (see representative compounds **3** and **4**, Figure 1). Here we unexpectedly found that *N*-methylation of some of the quinazoline analogues transformed quinazoline inhibitors into activators. We describe a broader SAR study of quinazoline derivatives to better understand the pharmacophoric requirements for this unusual activity switch as a result of such a minor structural change and to design novel noninhibitory modulators.

CHEMISTRY

The synthesis of target compounds for SAR exploration is similar to our previous synthesis route¹⁹ and is detailed in Schemes 1 and 2. As shown in Scheme 1, the intermediate 4-chloro-2-(pyridin-3-yl)quinazoline was prepared from 2-amino-benzonitrile and nicotinoyl chloride according to a published synthesis route²⁰. The reaction of 4-chloro-2-(pyridin-3-yl)quinazoline and appropriate amines in the presence of potassium carbonate as a base afforded **5a–5j** and **7a–7e**. Compounds **6a–6j**, **8a–8h**, and **9a–9v** were obtained either by deprotonation and *N*-methylation of the corresponding secondary amines (**5a–5j** and **7a–7e**), or by alkylation of 4-chloro-2-(pyridin-3-yl)quinazoline with appropriate secondary amines under basic conditions.

Additional analogues, having modifications at position 2 of the quinazoline ring, were synthesized by reaction of *N*-methyl-2-phenoxyethanamine with 2,4-dichloroquinazoline, followed by Suzuki coupling with appropriate boronic acids to afford **11b–11i** (Scheme 2). Compound **11a** was synthesized by reaction of commercially available 4-chloro-2-(pyridin-2-yl)quinazoline and *N*-methyl-2-phenoxyethanamine. The structure and purity of all of the prepared compounds were confirmed by spectroscopic and analytical techniques.

RESULTS AND DISCUSSION

Because of our unexpected observation that *N*-methylation of inhibitor **5a** to **6a** ($R^2 = \text{CH}_3$), transformed its activity into an activator (Figure 2), we explored a broader SAR of quinazoline derivatives in an attempt to uncover the pharmacophoric basis for this finding and to identify novel non-inhibitory modulators. First we examined a series of moderate to potent quinazoline inhibitors that we previously reported¹⁹ to see if they could also be switched to activators by *N*-methylation (Table 1). Interestingly, we were able to switch the moderately active inhibitors (**5a-5f**) to activators (**6a-6f**) by *N*-methylation, suggesting that these inhibitors and activators may share an allosteric binding site. According to our recent findings, the N-H forms a H-bond with Ser345 in the allosteric site²¹; this interaction may be important for attaining an inhibitory conformation, and *N*-methylation disrupted the H-bond and may change that conformation to one that promotes activation.

However, for the most potent inhibitors (**5g-5j**), *N*-methylation only produced weak inhibitory or inactive compounds (**6g-6j**). As we noted in our inhibitor SAR study,²⁰ the most potent inhibitors (**5h-5j**), having fused substituents (*R* groups), have additional hydrophobic interactions (and pi-pi stacking with Phe347) with loop 2 residues of GCa_v, which stabilizes the GCa_v dimer form. The removal of this presumed NH H-bond interaction with Ser345 is apparently not sufficient to completely change the conformation and/or break dimer formation so that these compounds retain partial inhibitory activity, and are not activators. Therefore, substituents of the secondary amines of inhibitors might also affect an activity switch.

To further explore the SAR, a methylene group in the phenylpropyl substituent of **5a** was modified to an oxygen atom to give ether **7a**, which was a moderate activator even without *N*-methylation (Figure 3). Compared to **7a**, the compounds with a longer substituent (**7b** and **7c**) or with a methyl group at the α - or β -position of **7a** (**7d** and **7e**) retained their inhibitory activity (Table 2). This finding suggests that the oxygen atom in **7a** may be involved in an intramolecular H-bond interaction with the NH group to avoid the intermolecular H-bond with Ser345, but an appropriate distance between the oxygen atom and quinazoline ring with no steric hindrance was required for proper H-bonding and to achieve this activation. Installation of an *N*-methyl group on the secondary amine of **7a** afforded **8a**, having the greatest maximum activation (E_{max} 761%) with an AC_{50} value (3.55 μM), similar to that of **7a** (Figure 3). Likewise, *N*-methylation of **7b-7e** switched these inhibitors to moderate activators **8b-8e** (Table 2). Activation of **8f-8h** was decreased with longer substituent or with increased steric hindrance of the carbon substituents (Table 2), suggesting a small hydrophobic pocket for the alkyl groups.

To further explore the conformation required for the activation activity, some conformationally-restricted substituents were introduced in the modulators (**9a-9k**, Table 3). As we expected, these compounds, without a free NH group, displayed activation activity (except for **9g** and **9h**). Interestingly, the chiral center of the stereoisomers (**9b** and **9c**) did not have a striking effect on the activity. We had previously demonstrated the important role of an aromatic ring in the *N*-substituents of our quinazoline inhibitors¹⁹; here we also observed

the key interaction of the aromatic ring in the activators. For example, compared to **9j**, compound **9k** with a fused phenyl ring gave a 5-fold lower AC₅₀ value.

To pursue potent activators with greater maximum activation, we further designed and synthesized derivatives **9l-9v** having a conformationally-restricted chain or a fused ring (Table 3). Most of the compounds showed similar activation activity as **8a**, except for **9n**, **9u**, and **9v**. Similar to **9b** and **9c**, compound **9q** has slightly better binding potency than its enantiomer (**9r**).

Finally, we examined the aromatic ring effect at position 2 of the quinazoline ring (Table 4). As we found in our SAR of quinazoline inhibitors, the 3-pyridinyl ring could be replaced by other aromatic rings, such as phenyl and 3-thienyl groups, without losing activity¹⁹. Unlike the inhibitors, the activator series modulators were not tolerant of modification of the 3-pyridinyl ring, except for **11b**, **11c**, and **11h**, which were comparable or enhanced in activity relative to **8a**. It should be noted that compound binding to the GCase allosteric site may not induce either inhibitory or activation activity, because the readout of compound activity was based on the enzyme activity. A binding-affinity based or competitive-binding assay may be developed for accurate evaluation of the compounds without inhibitory or activation activity.

To verify compound activity in cells, the most active compounds underwent preliminary screening in healthy control fibroblast cell assays. Compound **9q**, showing the most promising effect, was further tested in healthy control and compound heterozygous N370S/84GG *GBA1* mutant fibroblasts derived from a GD patient. Compound treatment increased the post-ER form (resistant to Endo H digestion) of wild-type GCase protein levels and improved enzyme activity at a concentration of 15 μ M in control fibroblasts, while control compound isofagomine (IFG) did not change post-ER GCase levels and enzyme activity at a concentration of 25 μ M (Figure 4A and Figure 5A). Similarly, we observed an increase of GCase protein levels and improved enzyme activity upon **9q** treatment in N370S/84GG *GBA1* mutant fibroblasts (Figure 4B and Figure 5B).

To confirm that the increase of GCase activity observed in fibroblast lysates occurred because of an increase in lysosomal GCase, we measured GCase activity in live cells. Fibroblasts from a healthy control and fibroblasts containing a homozygous N370S or L444P *GBA1* mutation were treated with **9q** and isofagomine and assayed for lysosomal GCase activity. Lysosomal specificity was achieved by calculating only the bafilomycin-sensitive hydrolysis of the substrate. We found that treatment with **9q** led to a significant, dose dependent, increase in lysosomal GCase activity in healthy control and *GBA1* mutant fibroblasts with a 55% and 85% increase in the 15 μ M treated N370S and L444P fibroblast, respectively (Figure 5C-E). In contrast, treatment with isofagomine showed little or no effect on GCase activity.

Similar effects were observed in induced pluripotent stem cell (iPSC)-derived healthy control neurons and heterozygous N370S *GBA1* mutant dopaminergic neurons from a PD patient (see Supporting Information Figure for the characterization of iPSCs and differentiated neurons) treated with increasing concentrations of **9q** for 10 days and digested with EndoH or PNGase. **9q** increased GCase protein levels and enzyme activity in both

wild-type and mutant neurons (Figure 6 and Figure 7), suggesting the potential application of these GCase modulators in PD patients with/without *GBA1* mutations.

CONCLUSIONS

Our SAR study revealed a remarkable finding that previously reported quinazoline inhibitors could be transformed to activators of GCase by *N*-methylation. Treatment of wild-type and *GBA1* mutant fibroblasts, as well as dopaminergic neurons with modulator **9q**, led to increased GCase protein levels and lysosomal enzyme activity, suggesting that further development of these compounds as GCase allosteric activators is warranted. It will be important to analyze the GCase-compound complex by crystallography to elucidate the mechanism of enzyme activation for future drug design.

EXPERIMENTAL SECTION

Chemistry

Materials, and Methods—Commercially available reagents and solvents were used without further purification. All reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm Silicycle extra hard 250 μ M TLC plates (60 F254). Purification of reaction products was carried out by flash chromatography using an Agilent 971-FP flash purification system with Silicycle silica gel columns. The yields are not optimized. The purity of all compounds was >95% as analyzed with an Agilent 1260 Infinity HPLC system. ^1H NMR and ^{13}C NMR spectra were obtained using a Bruker Avance III 500 MHz system (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) or Agilent DD MR-400 system (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) spectrometer. Chemical shifts are reported relative to chloroform ($\delta = 7.26$ for ^1H NMR and $\delta = 77.16$ for ^{13}C NMR spectra) or dimethyl sulfoxide ($\delta = 2.50$ for ^1H and $\delta = 39.52$ for ^{13}C NMR spectra). Data are reported as br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Mass spectra were obtained using a Bruker AmaZon SL system. High resolution mass spectra (HRMS) were performed using an Agilent 6210A LC-TOF instrument with a dual spray ESI source with a high resolution time of flight (TOF) mass analyzer and collecting in a 2GigHz detector mode coupled with an Agilent 1200 HPLC.

General Procedure for Compounds 5a-5j, 7a-7e, 9a-9v, and 11a.: A mixture of 4-chloro-2-(pyridin-3-yl)quinazoline^{19,22} (72 mg, 0.3 mmol), amine (0.3 mmol), and potassium carbonate (69 mg, 0.3 mmol) in DMF (2 mL) was stirred at room temperature or 60 oC overnight. Water (20 mL) was added, and the formed solid was filtered, washed with water, and dried *in vacuo* to give the products, which were usually pure (>95% purity). Those products without sufficient purity were further purified by flash chromatography.

General Procedure for Compounds 6a-6j and 8a-8h.: To a solution of **5a-5j** in dry DMF was added NaH (60% in mineral oil) under an argon atmosphere at room temperature, and the mixture was stirred for 15 min. Iodomethane or an appropriate halogenated alkane was added dropwise to the mixture, and the mixture was allowed to stir at room temperature overnight. Water was added, and the formed solid was filtered, washed with water, and dried *in vacuo* to give the desired product.

Preparation of 2-chloro-*N*-methyl-*N*-(2-phenoxyethyl)quinazolin-4-amine (10):

A mixture of 2,4-dichloroquinazoline (1.99 g, 10 mmol), *N*-methyl-2-phenoxyethanamine (1.51 g, 10 mmol), and potassium carbonate (1.38 g, 10 mmol) in DMF (20 mL) were stirred at room temperature overnight. Water (120 mL) was added, and the formed solid was filtered, washed with water, and dried to give **10** as a white solid (2.5 g, 80%).

^1H NMR (500 MHz, CDCl_3) δ 8.10 (d, J = 8.5 Hz, 1H), 7.77 (dd, J = 8.4, 0.8 Hz, 1H), 7.71 – 7.65 (m, 1H), 7.37 (ddd, J = 8.4, 7.0, 1.3 Hz, 1H), 7.30 – 7.25 (m, 2H), 6.97 – 6.91 (m, 3H), 4.38 (t, J = 5.5 Hz, 2H), 4.18 (t, J = 5.4 Hz, 2H), 3.61 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.7, 158.3, 156.1, 153.6, 133.0, 129.6, 127.8, 125.7, 124.8, 121.1, 114.4, 65.5, 52.6, 42.2. MS (ESI) m/z $[\text{M} + \text{H}]^+$: calcd, 314.11; found, 314.49.

General Procedure for Compounds 11b-11h: A mixture of 2-chloro-*N*-methyl-*N*-(2-phenoxyethyl)quinazolin-4-amine (**10**, 126 mg, 0.4 mmol), boronic acid (0.5 mmol), $\text{Pd}(\text{PPh}_3)_4$ (50 mg, 0.05 mmol), potassium carbonate (276 mg, 2.0 mmol) in dioxane (10 mL), and water (1.5 mL) was heated at 100 °C under an argon atmosphere for 16 h. Water (5 mL) was added, and the mixture was extracted with EtOAc (25 mL $\times$ 3). The combined organic phase was washed with brine (15 mL), dried (Na_2SO_4), filtered, evaporated, and purified by flash chromatography to give the product.

Analytical Data of the Novel Quinazoline Derivatives (6a-6j, 7a-7e, 8a-8h, 9a-9v, and 11a-11h)

***N*-Methyl-*N*-(3-phenylpropyl)-2-(pyridin-3-yl)quinazolin-4-amine (6a):** Yellow solid (yield: 72%). ^1H NMR (500 MHz, CDCl_3) δ 9.69 (s, 1H), 8.70 (dt, J = 7.9, 1.7 Hz, 1H), 8.66 (d, J = 4.4 Hz, 1H), 7.92 – 7.84 (m, 2H), 7.71 – 7.63 (m, 1H), 7.37 (dd, J = 7.9, 4.8 Hz, 1H), 7.31 – 7.25 (m, 3H), 7.21 – 7.16 (m, 3H), 3.86 – 3.80 (m, 2H), 3.41 (s, 3H), 2.74 (t, J = 7.5 Hz, 2H), 2.25 – 2.16 (m, 2H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.3, 157.3, 152.9, 150.6, 150.2, 141.2, 135.5, 134.3, 132.1, 128.7, 128.5, 128.4, 126.1, 125.2, 124.4, 123.1, 115.0, 52.7, 40.0, 33.3, 28.8. HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{23}\text{N}_4$ $[\text{M} + \text{H}]^+$, 355.1917; found, 355.1928.

***N*-Methyl-*N*-phenethyl-2-(pyridin-3-yl)quinazolin-4-amine (6b):** Pale-yellow solid (yield: 81%). ^1H NMR (500 MHz, CDCl_3) δ 9.74 (s, 1H), 8.80 – 8.56 (m, 2H), 7.94 (d, J = 8.2 Hz, 1H), 7.89 (d, J = 8.3 Hz, 1H), 7.70 – 7.62 (m, 1H), 7.41 – 7.30 (m, 2H), 7.30 – 7.16 (m, 5H), 4.07 – 3.97 (m, 2H), 3.39 (s, 3H), 3.17 – 3.07 (m, 2H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.0, 157.4, 152.9, 150.6, 150.1, 138.9, 135.6, 134.5, 132.2, 128.8, 128.7, 128.7, 126.5, 125.3, 124.5, 123.2, 115.0, 54.9, 40.7, 33.6. HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{21}\text{N}_4$ $[\text{M} + \text{H}]^+$, 341.1761; found, 341.1770.

***N*-Methyl-*N*-(4-phenylbutyl)-2-(pyridin-3-yl)quinazolin-4-amine (6c):** Pale-yellow oil (yield: 67%). ^1H NMR (500 MHz, CDCl_3) δ 9.72 (s, 1H), 8.75 (d, J = 7.9 Hz, 1H), 8.68 (s, 1H), 7.94 (d, J = 7.7 Hz, 1H), 7.91 (d, J = 8.3 Hz, 1H), 7.71 – 7.66 (m, 1H), 7.42 – 7.33 (m, 2H), 7.28 – 7.23 (t, J = 7.6 Hz, 2H), 7.19 – 7.12 (m, 3H), 3.85 – 3.79 (m, 2H), 3.39 (s, 3H), 2.68 (t, J = 7.6 Hz, 2H), 1.93 – 1.84 (m, 2H), 1.78 – 1.70 (m, 2H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.3, 157.4, 152.9, 150.5, 150.1, 141.9, 135.6, 134.4, 132.2, 128.7, 128.3, 125.9,

125.3, 124.4, 123.3, 115.1, 53.1, 40.0, 35.6, 28.7, 26.9. HRMS (ESI): calcd for $C_{24}H_{25}N_4$ $[M+H]^+$, 369.2074; found, 369.2080.

N-Cyclopentyl-N-methyl-2-(pyridin-3-yl)quinazolin-4-amine (6d).: Yellow solid (yield: 76%). 1H NMR (500 MHz, $CDCl_3$) δ 9.71 (s, 1H), 8.77 (dt, J = 7.9, 1.8 Hz, 1H), 8.65 (dd, J = 4.7, 1.5 Hz, 1H), 7.98 (d, J = 8.3 Hz, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.68 (t, J = 7.1 Hz, 1H), 7.44 – 7.31 (m, 2H), 5.04 – 4.92 (m, 1H), 3.25 (s, 3H), 2.15 – 2.02 (m, 2H), 1.86 – 1.60 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.4, 157.4, 152.9, 150.5, 150.2, 135.6, 134.4, 132.1, 128.6, 125.4, 124.4, 123.1, 115.5, 61.6, 34.4, 28.8, 24.6. HRMS (ESI): calcd for $C_{19}H_{21}N_4$ $[M+H]^+$, 305.1761; found, 305.1762.

N-Cyclohexyl-N-methyl-2-(pyridin-3-yl)quinazolin-4-amine (6e).: Pale-yellow solid (yield: 59%). 1H NMR (500 MHz, $CDCl_3$) δ 9.72 (s, 1H), 8.79 (d, J = 7.8 Hz, 1H), 8.67 (d, J = 3.8 Hz, 1H), 7.94 (d, J = 8.3 Hz, 2H), 7.70 (t, J = 7.6 Hz, 1H), 7.43 – 7.33 (m, 2H), 4.58 – 4.46 (m, 1H), 3.28 (s, 3H), 2.01 (d, J = 11.5 Hz, 2H), 1.92 (d, J = 13.2 Hz, 2H), 1.79 – 1.63 (m, 3H), 1.53 – 1.41 (m, 2H), 1.26 – 1.15 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.8, 157.3, 153.0, 150.7, 150.3, 135.7, 134.4, 132.2, 128.7, 125.5, 124.4, 123.2, 115.4, 59.9, 34.3, 30.1, 26.0, 25.8. HRMS (ESI): calcd for $C_{20}H_{23}N_4$ $[M+H]^+$, 319.1917; found, 319.1921.

N-Cycloheptyl-N-methyl-2-(pyridin-3-yl)quinazolin-4-amine (6f).: Pale-yellow solid (yield: 72%). 1H NMR (500 MHz, $CDCl_3$) δ 9.71 (s, 1H), 8.77 (d, J = 7.9 Hz, 1H), 8.65 (d, J = 3.5 Hz, 1H), 7.94 (d, J = 8.3 Hz, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.73 – 7.60 (m, 1H), 7.44 – 7.31 (m, 2H), 4.77 – 4.65 (m, 1H), 3.27 (s, 3H), 2.13 – 2.02 (m, 2H), 1.93 – 1.74 (m, 4H), 1.74 – 1.52 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.4, 157.4, 153.0, 150.5, 150.2, 135.6, 134.5, 132.0, 128.6, 125.4, 124.2, 123.1, 115.3, 61.2, 34.5, 32.2, 27.7, 25.5. HRMS (ESI): calcd for $C_{21}H_{25}N_4$ $[M+H]^+$, 333.2074; found, 333.2082.

N-Cyclooctyl-N-methyl-2-(pyridin-3-yl)quinazolin-4-amine (6g).: Yellow solid (yield: 86%). 1H NMR (500 MHz, $CDCl_3$) δ 9.71 (d, J = 1.6 Hz, 1H), 8.77 (dt, J = 7.9, 1.9 Hz, 1H), 8.65 (dd, J = 4.7, 1.6 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 8.3 Hz, 1H), 7.72 – 7.63 (m, 1H), 7.41 – 7.31 (m, 2H), 4.99 – 4.87 (m, 1H), 3.27 (s, 3H), 2.02 – 1.87 (m, 4H), 1.87 – 1.77 (m, 2H), 1.77 – 1.52 (m, 8H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.2, 157.4, 153.0, 150.5, 150.2, 135.6, 134.5, 132.0, 128.6, 125.5, 124.2, 123.1, 115.3, 59.5, 34.7, 31.6, 29.7, 26.4, 25.2. HRMS (ESI): calcd for $C_{22}H_{27}N_4$ $[M+H]^+$, 347.2230; found, 347.2238.

N-(2,3-Dihydro-1H-inden-2-yl)-N-methyl-2-(pyridin-3-yl)quinazolin-4-amine (6h).: Yellow solid (yield: 60%). 1H NMR (500 MHz, $CDCl_3$) δ 9.74 (d, J = 1.5 Hz, 1H), 8.77 (dt, J = 7.9, 1.9 Hz, 1H), 8.68 (dd, J = 4.7, 1.5 Hz, 1H), 8.04 (d, J = 7.9 Hz, 1H), 7.96 (d, J = 8.3 Hz, 1H), 7.78 – 7.69 (m, 1H), 7.45 – 7.41 (m, 1H), 7.39 (dd, J = 7.9, 4.8 Hz, 1H), 7.29 (dd, J = 5.2, 3.4 Hz, 2H), 7.25 – 7.20 (m, 2H), 5.66 – 5.58 (m, 1H), 3.47 (dd, J = 16.4, 8.6 Hz, 2H), 3.35 – 3.26 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.7, 157.5, 153.1, 150.8, 150.4, 141.3, 135.7, 134.3, 132.5, 129.0, 126.9, 125.5, 124.8, 124.7, 123.3, 115.7, 60.2, 36.4, 35.6. HRMS (ESI): calcd for $C_{23}H_{21}N_4$ $[M+H]^+$, 353.1761; found, 353.1772.

(S)-N-Methyl-2-(pyridin-3-yl)-N-(1,2,3,4-tetrahydronaphthalen-2-yl)quinazolin-4-amine (6i).: Pale-yellow oil (yield: 63%). 1H NMR (500 MHz, $CDCl_3$) δ 9.70 (s, 1H), 8.77 (dt, J =

7.9, 1.9 Hz, 1H), 8.65 (s, 1H), 7.97 (d, $J = 7.7$ Hz, 1H), 7.93 (d, $J = 7.7$ Hz, 1H), 7.77 – 7.63 (m, 1H), 7.45 – 7.30 (m, 2H), 7.16–7.10 (m, 4H), 5.07 – 4.90 (m, 1H), 3.34 (s, 3H), 3.21 (d, $J = 8.4$ Hz, 2H), 3.12 – 2.91 (m, 2H), 2.29 – 2.17 (m, 1H), 2.11 (qd, $J = 11.9, 5.9$ Hz, 1H). ^{13}C NMR (125 MHz, CDCl_3) δ 164.1, 157.3, 153.0, 150.6, 150.2, 135.6, 135.6, 135.2, 134.3, 132.3, 129.4, 128.8, 126.1, 126.0, 125.3, 124.6, 123.2, 115.4, 56.5, 34.2, 32.0, 29.5, 27.1. HRMS (ESI): calcd for $\text{C}_{24}\text{H}_{23}\text{N}_4$ $[\text{M}+\text{H}]^+$, 367.1917; found, 367.1932.

(R)-N-Methyl-2-(pyridin-3-yl)-N-(1,2,3,4-tetrahydronaphthalen-2-yl)quinazolin-4-amine (6j).: Pale-yellow oil (yield: 69%). ^1H NMR (500 MHz, CDCl_3) δ 9.70 (s, 1H), 8.77 (dt, $J = 7.9, 1.9$ Hz, 1H), 8.65 (s, 1H), 7.97 (d, $J = 7.7$ Hz, 1H), 7.93 (d, $J = 7.7$ Hz, 1H), 7.77 – 7.63 (m, 1H), 7.45 – 7.30 (m, 2H), 7.16–7.10 (m, 4H), 5.07 – 4.90 (m, 1H), 3.34 (s, 3H), 3.21 (d, $J = 8.4$ Hz, 2H), 3.12 – 2.91 (m, 2H), 2.29 – 2.17 (m, 1H), 2.11 (qd, $J = 11.9, 5.9$ Hz, 1H). ^{13}C NMR (125 MHz, CDCl_3) δ 164.1, 157.3, 153.0, 150.6, 150.2, 135.6, 135.6, 135.2, 134.3, 132.3, 129.4, 128.8, 126.1, 126.0, 125.3, 124.6, 123.2, 115.4, 56.5, 34.2, 32.0, 29.5, 27.1. HRMS (ESI): calcd for $\text{C}_{24}\text{H}_{23}\text{N}_4$ $[\text{M}+\text{H}]^+$, 367.1917; found, 367.1927.

N-(2-Phenoxyethyl)-2-(pyridin-3-yl)quinazolin-4-amine (7a).: Off-white solid (yield: 67%). ^1H NMR (500 MHz, CDCl_3) δ 9.75 (d, $J = 1.6$ Hz, 1H), 8.81 (dt, $J = 7.9, 1.9$ Hz, 1H), 8.70 (dd, $J = 4.8, 1.6$ Hz, 1H), 7.93 (d, $J = 8.2$ Hz, 1H), 7.79 – 7.73 (m, 2H), 7.50 – 7.46 (m, 1H), 7.42 (dd, $J = 7.9, 4.8$ Hz, 1H), 7.34 – 7.28 (m, 2H), 7.01 – 6.95 (m, 3H), 6.28 – 6.17 (m, 1H), 4.39 – 4.29 (m, 2H), 4.28 – 4.23 (m, 2H). ^{13}C NMR (125 MHz, CDCl_3) δ 159.7, 158.6, 150.8, 150.4, 150.3, 135.7, 134.4, 132.9, 129.7, 129.0, 126.0, 123.3, 121.3, 120.8, 114.6, 114.0, 66.4, 40.8. HRMS (ESI): calcd for $\text{C}_{21}\text{H}_{19}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 343.1553; found, 343.1564.

N-Methyl-N-(2-phenoxyethyl)-2-(pyridin-3-yl)quinazolin-4-amine (8a).: Off-white solid (yield: 96%). ^1H NMR (500 MHz, CDCl_3) δ 9.69 (s, 1H), 8.75 (dt, $J = 7.9, 1.9$ Hz, 1H), 8.67 (d, $J = 3.5$ Hz, 1H), 8.13 (d, $J = 8.5$ Hz, 1H), 7.92 (d, $J = 8.4$ Hz, 1H), 7.71 (ddd, $J = 8.3, 7.0, 1.3$ Hz, 1H), 7.44 – 7.34 (m, 2H), 7.28 – 7.24 (m, 2H), 6.96 – 6.87 (m, 3H), 4.45 (t, $J = 5.4$ Hz, 2H), 4.28 (t, $J = 5.4$ Hz, 2H), 3.63 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.1, 158.5, 157.2, 153.0, 150.7, 150.1, 135.5, 134.2, 132.3, 129.5, 128.8, 125.5, 124.6, 123.2, 121.1, 115.1, 114.5, 65.9, 52.5, 41.9. HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{21}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 357.1710; found, 357.1722.

N-(2-(Benzyloxy)ethyl)-2-(pyridin-3-yl)quinazolin-4-amine (7b).: Pale-yellow solid (51%). ^1H NMR (500 MHz, CDCl_3) δ 9.72 (s, 1H), 8.77 (d, $J = 7.9$ Hz, 1H), 8.68 (s, 1H), 7.90 (d, $J = 8.3$ Hz, 1H), 7.78 – 7.71 (m, 1H), 7.68 (d, $J = 8.1$ Hz, 1H), 7.46 – 7.41 (m, 1H), 7.41 – 7.37 (m, 1H), 7.36 – 7.30 (m, 4H), 6.27 – 6.16 (m, 1H), 4.59 (s, 2H), 4.01 (q, $J = 5.2$ Hz, 2H), 3.84 – 3.80 (m, 2H). ^{13}C NMR (125 MHz, CDCl_3) δ 159.6, 158.5, 150.6, 150.2, 150.1, 137.8, 135.6, 132.7, 128.8, 128.5, 127.9, 127.8, 125.8, 123.2, 120.7, 113.9, 73.3, 68.4, 41.0. HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{21}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 357.1710; found, 357.1721.

N-(2-(Benzyloxy)ethyl)-N-methyl-2-(pyridin-3-yl)quinazolin-4-amine (8b).: Pale-yellow oil (87%). ^1H NMR (500 MHz, CDCl_3) δ 9.63 (s, 1H), 8.68 (dt, $J = 7.9, 1.9$ Hz, 1H), 8.61 (d, $J = 3.7$ Hz, 1H), 8.07 (dd, $J = 8.4, 0.8$ Hz, 1H), 7.85 (dd, $J = 8.4, 0.8$ Hz, 1H), 7.63 (ddd, $J = 8.3, 7.0, 1.3$ Hz, 1H), 7.34 – 7.28 (m, 2H), 7.24 – 7.16 (m, 5H), 4.50 (s, 2H), 4.01 (t, $J = 5.5$

Hz, 2H), 3.88 (t, J = 5.5 Hz, 2H), 3.46 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.2, 157.2, 152.9, 150.6, 150.1, 138.0, 135.5, 134.3, 132.2, 128.7, 128.4, 127.7, 127.5, 125.6, 124.5, 123.1, 115.1, 73.3, 68.1, 53.1, 41.2. HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 371.1866; found, 371.1880.

***N*-(3-Phenoxypropyl)-2-(pyridin-3-yl)quinazolin-4-amine (7c).**: Off-white solid (yield: 73%). ^1H NMR (500 MHz, CDCl_3) δ 9.75 (s, 1H), 8.79 (d, J = 7.9 Hz, 1H), 8.66 (d, J = 3.4 Hz, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.77 – 7.63 (m, 2H), 7.47 – 7.39 (m, 1H), 7.37 (dd, J = 7.8, 4.8 Hz, 1H), 7.32 – 7.25 (m, 2H), 6.99 – 6.90 (m, 3H), 6.50 (s, 1H), 4.18 (t, J = 5.6 Hz, 2H), 4.02 – 3.95 (m, 2H), 2.28 (p, J = 5.9 Hz, 2H). ^{13}C NMR (125 MHz, CDCl_3) δ 159.7, 158.6, 158.5, 150.6, 150.2, 150.2, 135.6, 134.5, 132.7, 129.6, 128.8, 125.8, 123.1, 121.1, 120.6, 114.4, 113.9, 66.8, 39.7, 28.5. HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{21}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 357.1710; found, 357.1723.

***N*-Methyl-*N*-(3-phenoxypropyl)-2-(pyridin-3-yl)quinazolin-4-amine (8c).**: Yellow solid (77%). ^1H NMR (500 MHz, CDCl_3) δ 9.72 (s, 1H), 8.77 (dt, J = 7.9, 1.9 Hz, 1H), 8.66 (d, J = 3.7 Hz, 1H), 8.06 (dd, J = 8.4, 0.8 Hz, 1H), 7.91 (dd, J = 8.4, 0.8 Hz, 1H), 7.70 (ddd, J = 8.3, 7.0, 1.3 Hz, 1H), 7.42 – 7.33 (m, 2H), 7.29 – 7.24 (m, 2H), 6.93 (t, J = 7.3 Hz, 1H), 6.88 (d, J = 7.8 Hz, 2H), 4.10 (t, J = 5.8 Hz, 2H), 4.07 – 4.02 (m, 2H), 3.48 (s, 3H), 2.40 – 2.32 (m, 2H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.2, 158.7, 157.3, 152.9, 150.6, 150.1, 135.6, 134.3, 132.2, 129.5, 128.8, 125.4, 124.5, 123.1, 120.8, 115.1, 114.4, 65.2, 50.5, 40.5, 27.2. HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 371.1866; found, 371.1879.

***N*-(2-Phenoxypropyl)-2-(pyridin-3-yl)quinazolin-4-amine (7d).**: Off-white solid (yield: 34%). ^1H NMR (500 MHz, CDCl_3) δ 9.76 (s, 1H), 8.79 (d, J = 7.9 Hz, 1H), 8.71 (s, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.75 (ddd, J = 8.3, 7.0, 1.2 Hz, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.50 – 7.36 (m, 2H), 7.31 – 7.26 (m, 2H), 7.02 – 6.91 (m, 3H), 6.35 – 6.20 (m, 1H), 4.87 – 4.75 (m, 1H), 4.31 (ddd, J = 13.9, 6.8, 3.5 Hz, 1H), 3.85 (ddd, J = 13.9, 7.5, 4.7 Hz, 1H), 1.44 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 159.6, 158.5, 157.6, 150.7, 150.3, 150.2, 135.6, 134.3, 132.8, 129.7, 128.9, 126.0, 123.2, 121.4, 120.6, 116.2, 113.8, 72.8, 46.1, 17.6. HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{21}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 357.1710; found, 357.1720.

***N*-Methyl-*N*-(2-phenoxypropyl)-2-(pyridin-3-yl)quinazolin-4-amine (8d).**: Off-white solid (yield: 67%). ^1H NMR (500 MHz, CDCl_3) δ 9.67 (s, 1H), 8.74 (dt, J = 7.9, 1.7 Hz, 1H), 8.67 (d, J = 3.8 Hz, 1H), 8.04 (d, J = 8.3 Hz, 1H), 7.91 (d, J = 8.2 Hz, 1H), 7.73 – 7.65 (m, 1H), 7.38 (dd, J = 7.9, 4.8 Hz, 1H), 7.37 – 7.32 (m, 1H), 7.21 – 7.13 (m, 2H), 6.91 – 6.79 (m, 3H), 5.06 – 4.97 (m, 1H), 4.36 (dd, J = 14.1, 3.2 Hz, 1H), 3.90 (dd, J = 14.1, 8.1 Hz, 1H), 3.59 (s, 3H), 1.42 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.0, 157.8, 157.1, 153.0, 150.6, 150.0, 135.6, 134.3, 132.3, 129.5, 128.6, 125.6, 124.5, 123.2, 121.0, 115.7, 115.0, 72.9, 58.4, 42.9, 18.1. HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 371.1866; found, 371.1879.

***N*-(1-Phenoxypropan-2-yl)-2-(pyridin-3-yl)quinazolin-4-amine (7e).**: Pale-yellow solid (yield: 60%). ^1H NMR (500 MHz, CDCl_3) δ 10.01 (s, 1H), 9.08 (d, J = 7.9 Hz, 1H), 8.96 (s, 1H), 8.21 (d, J = 8.2 Hz, 1H), 8.09 – 7.96 (m, 2H), 7.76 – 7.65 (m, 2H), 7.58 – 7.50 (m, 3H), 7.26 – 7.21 (m, 2H), 6.44 (d, J = 7.1 Hz, 1H), 5.39 – 5.29 (m, 1H), 4.51 (dd, J = 9.4, 4.3 Hz,

1H), 4.46 (dd, $J = 9.3, 3.7$ Hz, 1H), 1.84 (d, $J = 6.8$ Hz, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 159.0, 158.6, 158.4, 150.5, 150.1, 150.0, 135.8, 134.3, 132.9, 129.6, 128.7, 126.0, 123.3, 121.3, 120.7, 114.6, 113.8, 70.3, 46.2, 17.5. HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{21}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 357.1710; found, 357.1726.

N-Methyl-N-(1-phenoxypropan-2-yl)-2-(pyridin-3-yl)quinazolin-4-amine (8e).: Pale-yellow oil (yield: 78%). ^1H NMR (500 MHz, CDCl_3) δ 9.71 (s, 1H), 8.76 (d, $J = 7.9$ Hz, 1H), 8.67 (s, 1H), 8.06 (d, $J = 8.4$ Hz, 1H), 7.93 (d, $J = 8.4$ Hz, 1H), 7.73 – 7.66 (m, 1H), 7.43 – 7.32 (m, 2H), 7.22 (dd, $J = 8.6, 7.5$ Hz, 2H), 6.91 (t, $J = 7.4$ Hz, 1H), 6.84 (d, $J = 7.8$ Hz, 2H), 5.27 – 5.16 (m, 1H), 4.28 (dd, $J = 9.8, 7.6$ Hz, 1H), 4.16 (dd, $J = 9.9, 5.1$ Hz, 1H), 3.34 (s, 3H), 1.51 (d, $J = 6.9$ Hz, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 164.5, 158.4, 157.3, 153.0, 150.6, 150.2, 135.6, 134.3, 132.3, 129.5, 128.7, 125.4, 124.6, 123.2, 121.1, 115.4, 114.6, 69.2, 54.4, 33.9, 14.3. HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 371.1866; found, 371.1882.

N-Ethyl-N-(2-phenoxyethyl)-2-(pyridin-3-yl)quinazolin-4-amine (8f).: Off-white solid (yield: 68%). ^1H NMR (500 MHz, CDCl_3) δ 9.69 (d, $J = 1.5$ Hz, 1H), 8.74 (dt, $J = 7.9, 1.9$ Hz, 1H), 8.67 (dd, $J = 4.8, 1.6$ Hz, 1H), 8.00 (d, $J = 8.4$ Hz, 1H), 7.93 (d, $J = 8.4$ Hz, 1H), 7.74 – 7.67 (m, 1H), 7.43 – 7.34 (m, 2H), 7.26 – 7.22 (m, 2H), 6.95 – 6.87 (m, 3H), 4.42 (t, $J = 5.6$ Hz, 2H), 4.21 (t, $J = 5.6$ Hz, 2H), 3.94 (q, $J = 7.1$ Hz, 2H), 1.53 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 162.8, 158.5, 157.2, 152.9, 150.7, 150.1, 135.5, 134.2, 132.3, 129.5, 129.0, 124.9, 124.8, 123.2, 121.0, 115.2, 114.5, 65.7, 49.7, 47.7, 13.7. HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 371.1866; found, 371.1882.

N-(2-Phenoxyethyl)-N-propyl-2-(pyridin-3-yl)quinazolin-4-amine (8g).: Pale-yellow solid (yield: 49%). ^1H NMR (500 MHz, CDCl_3) δ 9.68 (d, $J = 1.7$ Hz, 1H), 8.74 (dt, $J = 7.9, 1.7$ Hz, 1H), 8.66 (dd, $J = 4.8, 1.5$ Hz, 1H), 7.97 (d, $J = 8.4$ Hz, 1H), 7.93 (d, $J = 8.3$ Hz, 1H), 7.74 – 7.66 (m, 1H), 7.43 – 7.35 (m, 2H), 7.27 – 7.21 (m, 2H), 6.95 – 6.87 (m, 3H), 4.41 (t, $J = 5.6$ Hz, 2H), 4.22 (t, $J = 5.6$ Hz, 2H), 3.84 – 3.78 (m, 2H), 2.02 – 1.93 (m, 2H), 1.02 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 162.9, 158.5, 157.2, 152.9, 150.7, 150.1, 135.5, 134.2, 132.3, 129.5, 129.0, 124.9, 124.7, 123.2, 121.0, 115.2, 114.5, 65.7, 54.8, 50.4, 21.6, 11.3. HRMS (ESI): calcd for $\text{C}_{24}\text{H}_{25}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 385.2023; found, 385.2039.

N-(Cyclopropylmethyl)-N-(2-phenoxyethyl)-2-(pyridin-3-yl)quinazolin-4-amine (8h).: Off-white solid (33%). ^1H NMR (500 MHz, CDCl_3) δ 9.70 (d, $J = 1.5$ Hz, 1H), 8.75 (dt, $J = 7.9, 1.8$ Hz, 1H), 8.67 (dd, $J = 4.7, 1.4$ Hz, 1H), 8.10 (d, $J = 8.4$ Hz, 1H), 7.93 (d, $J = 8.4$ Hz, 1H), 7.78 – 7.65 (m, 1H), 7.45 – 7.34 (m, 2H), 7.23 (t, $J = 15.9$ Hz, 2H), 6.92 (t, $J = 7.3$ Hz, 1H), 6.88 (d, $J = 8.0$ Hz, 2H), 4.43 (t, $J = 5.6$ Hz, 2H), 4.34 (t, $J = 5.6$ Hz, 2H), 3.79 (d, $J = 6.3$ Hz, 2H), 1.36 – 1.26 (m, 1H), 0.70 – 0.63 (m, 2H), 0.38 – 0.32 (m, 2H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.3, 158.5, 157.2, 152.9, 150.7, 150.1, 135.6, 134.2, 132.3, 129.5, 129.0, 125.1, 124.9, 123.2, 121.0, 115.3, 114.5, 65.9, 57.2, 49.6, 10.1, 4.0. HRMS (ESI): calcd for $\text{C}_{25}\text{H}_{25}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 397.2023; found, 397.2043.

4-(3-Phenoxypropyl)-2-(pyridin-3-yl)quinazoline (9a).: Pale-yellow solid (yield: 31%). ^1H NMR (500 MHz, CDCl_3) δ 9.71 (d, $J = 1.5$ Hz, 1H), 8.76 (dt, $J = 7.9, 1.9$ Hz, 1H), 8.65 (dd, $J = 4.8, 1.7$ Hz, 1H), 8.17 (d, $J = 8.3$ Hz, 1H), 7.91 (dd, $J = 8.3, 0.7$ Hz, 1H),

7.72 – 7.67 (m, 1H), 7.41 – 7.35 (m, 2H), 7.33 – 7.28 (m, 2H), 6.98 (t, $J = 7.4$ Hz, 1H), 6.93 (d, $J = 7.9$ Hz, 2H), 5.09–5.13 (m, 1H), 4.39 – 4.27 (m, 2H), 4.27 – 4.14 (m, 2H), 2.54 – 2.40 (m, 1H), 2.33 – 2.17 (m, 1H). ^{13}C NMR (125 MHz, CDCl_3) δ 160.0, 157.8, 156.9, 152.5, 150.6, 150.2, 135.5, 134.3, 132.2, 129.7, 128.6, 125.1, 124.6, 123.0, 121.4, 115.6, 115.3, 75.1, 55.9, 48.8, 31.4. HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{21}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 369.1710; found, 369.1727.

(S)-4-(3-Phenoxypiperidin-1-yl)-2-(pyridin-3-yl)quinazoline (9b).: Yellow oil (yield: 79%). ^1H NMR (500 MHz, CDCl_3) δ 9.71 (d, $J = 1.4$ Hz, 1H), 8.73 (dt, $J = 7.9, 1.9$ Hz, 1H), 8.68 (dd, $J = 4.8, 1.7$ Hz, 1H), 7.94 (s, 1H), 7.70 (ddd, $J = 8.3, 7.0, 1.3$ Hz, 1H), 7.42 – 7.33 (m, 2H), 7.30 – 7.25 (m, 2H), 6.98 – 6.92 (m, 3H), 4.64 – 4.55 (m, 1H), 4.36 (dd, $J = 13.1, 3.2$ Hz, 1H), 4.04 – 3.95 (m, 1H), 3.76 – 3.61 (m, 2H), 2.24 – 2.16 (m, 1H), 2.13 – 2.04 (m, 1H), 1.97 – 1.88 (m, 1H), 1.84 – 1.73 (m, 2H). ^{13}C NMR (125 MHz, CDCl_3) δ 165.0, 157.7, 157.2, 152.7, 150.7, 150.2, 135.6, 134.2, 132.6, 129.6, 128.9, 125.2, 125.0, 123.1, 121.3, 116.0, 115.4, 71.4, 53.4, 50.3, 30.1, 22.9. HRMS (ESI): calcd for $\text{C}_{24}\text{H}_{23}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 383.1866; found, 383.1881.

(R)-4-(3-Phenoxypiperidin-1-yl)-2-(pyridin-3-yl)quinazoline (9c).: Yellow oil (yield: 63%). ^1H NMR (500 MHz, CDCl_3) δ 9.71 (d, $J = 1.4$ Hz, 1H), 8.73 (dt, $J = 7.9, 1.9$ Hz, 1H), 8.68 (dd, $J = 4.8, 1.7$ Hz, 1H), 7.94 (s, 1H), 7.70 (ddd, $J = 8.3, 7.0, 1.3$ Hz, 1H), 7.42 – 7.33 (m, 2H), 7.30 – 7.25 (m, 2H), 6.98 – 6.92 (m, 3H), 4.64 – 4.55 (m, 1H), 4.36 (dd, $J = 13.1, 3.2$ Hz, 1H), 4.04 – 3.95 (m, 1H), 3.76 – 3.61 (m, 2H), 2.24 – 2.16 (m, 1H), 2.13 – 2.04 (m, 1H), 1.97 – 1.88 (m, 1H), 1.84 – 1.73 (m, 2H). ^{13}C NMR (125 MHz, CDCl_3) δ 165.0, 157.7, 157.2, 152.7, 150.7, 150.2, 135.6, 134.2, 132.6, 129.6, 128.9, 125.2, 125.0, 123.1, 121.3, 116.0, 115.4, 71.4, 53.4, 50.3, 30.1, 22.9. HRMS (ESI): calcd for $\text{C}_{24}\text{H}_{23}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 383.1866; found, 383.1883.

4-(2-Benzylpiperidin-1-yl)-2-(pyridin-3-yl)quinazoline (9d).: Yellow solid (yield: 42%). ^1H NMR (500 MHz, CDCl_3) δ 9.72 (s, 1H), 8.76 (d, $J = 7.9$ Hz, 1H), 8.69 (d, $J = 4.6$ Hz, 1H), 7.90 (d, $J = 8.4$ Hz, 1H), 7.73 (d, $J = 8.3$ Hz, 1H), 7.70 (d, $J = 7.0$ Hz, 1H), 7.40 (dd, $J = 7.8, 4.9$ Hz, 1H), 7.36 (d, $J = 7.1$ Hz, 1H), 7.22 – 7.15 (m, 4H), 7.12 (d, $J = 6.4$ Hz, 1H), 5.00 – 5.06 (m, 1H), 4.30 (d, $J = 13.2$ Hz, 1H), 3.68 – 3.55 (m, 1H), 3.26 (dd, $J = 13.5, 6.6$ Hz, 1H), 3.09 (dd, $J = 13.5, 8.8$ Hz, 1H), 2.01 – 1.67 (m, 6H). ^{13}C NMR (125 MHz, CDCl_3) δ 165.2, 157.7, 152.9, 150.7, 150.4, 139.0, 135.7, 134.5, 132.4, 129.2, 128.8, 128.5, 126.4, 125.1, 124.7, 123.2, 115.6, 58.0, 45.4, 35.8, 27.3, 25.9, 19.5. HRMS (ESI): calcd for $\text{C}_{25}\text{H}_{25}\text{N}_4$ $[\text{M}+\text{H}]^+$, 381.2074; found, 381.2084.

4-(3-Benzylpiperidin-1-yl)-2-(pyridin-3-yl)quinazoline (9e).: Off-white solid (yield: 46%). ^1H NMR (500 MHz, CDCl_3) δ 9.70 (s, 1H), 8.68 (d, $J = 7.6$ Hz, 2H), 7.92 (d, $J = 8.2$ Hz, 1H), 7.71 – 7.59 (m, 2H), 7.44 – 7.36 (m, 1H), 7.36 – 7.29 (m, 2H), 7.27 – 7.23 (m, 2H), 7.21 (d, $J = 7.1$ Hz, 2H), 4.43 (dd, $J = 30.6, 13.0$ Hz, 2H), 3.30 – 3.14 (m, 1H), 2.91 (dd, $J = 13.0, 10.7$ Hz, 1H), 2.69 (dd, $J = 13.5, 6.5$ Hz, 1H), 2.60 (dd, $J = 13.5, 8.1$ Hz, 1H), 2.24 – 2.08 (m, 1H), 2.06 – 1.95 (m, 1H), 1.95 – 1.84 (m, 1H), 1.84 – 1.67 (m, 1H), 1.48 – 1.30 (m, 1H). ^{13}C NMR (125 MHz, CDCl_3) δ 164.6, 157.6, 152.7, 150.7, 150.3, 139.8, 135.7, 132.5,

129.1, 128.8, 128.6, 126.3, 125.1, 125.0, 123.3, 115.5, 55.6, 50.6, 40.7, 38.6, 31.7, 25.7.
HRMS (ESI): calcd for $C_{25}H_{25}N_4$ $[M+H]^+$, 381.2074; found, 381.2089.

4-(3-Phenylpiperidin-1-yl)-2-(pyridin-3-yl)quinazoline (9f).: Yellow solid (yield: 34%). 1H NMR (500 MHz, $CDCl_3$) δ 9.68 (s, 1H), 8.73 (d, J = 7.9 Hz, 1H), 8.61 (d, J = 3.9 Hz, 1H), 7.87 (dd, J = 17.0, 8.3 Hz, 2H), 7.66 (s, 1H), 7.38 – 7.27 (m, 5H), 7.25 – 7.18 (m, 2H), 4.58 – 4.45 (m, 2H), 3.24 – 3.13 (m, 2H), 3.08 – 2.98 (m, 1H), 2.12 (d, J = 12.3 Hz, 1H), 1.96 – 1.73 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.0, 157.7, 152.8, 150.8, 150.3, 143.5, 135.7, 134.3, 132.6, 129.0, 128.8, 127.2, 126.9, 125.3, 125.2, 123.2, 115.8, 56.6, 50.8, 42.8, 32.2, 25.9. HRMS (ESI): calcd for $C_{24}H_{23}N_4$ $[M+H]^+$, 367.1917; found, 367.1930.

4-(3,4-Dihydroisoquinolin-2(1H)-yl)-2-(pyridin-3-yl)quinazoline (9g).: Yellow solid (yield: 44%). 1H NMR (400 MHz, $CDCl_3$) δ 9.79 (s, 1H), 8.83 (d, J = 7.9 Hz, 1H), 8.71 (s, 1H), 7.99 (dd, J = 14.1, 8.4 Hz, 2H), 7.81 – 7.69 (m, 1H), 7.53 – 7.37 (m, 2H), 7.23 (s, 4H), 5.07 (s, 2H), 4.15 (t, J = 5.7 Hz, 2H), 3.22 (t, J = 5.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 157.6, 152.8, 150.8, 150.4, 135.8, 134.8, 133.9, 132.6, 129.0, 128.9, 126.9, 126.6, 126.5, 125.2, 125.0, 123.3, 115.6, 51.4, 48.2, 29.0. HRMS (ESI): calcd for $C_{22}H_{19}N_4$ $[M+H]^+$, 339.1604; found, 339.1613.

2-(2-(pyridin-3-yl)quinazolin-4-yl)-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (9h).: Light-brown solid (yield: 37%). 1H NMR (500 MHz, $CDCl_3$) δ 9.78 (s, 1H), 8.81 (d, J = 7.9 Hz, 1H), 8.69 (d, J = 3.7 Hz, 1H), 8.21 (s, 1H), 8.04 (d, J = 8.2 Hz, 1H), 7.94 (d, J = 8.3 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.48 (t, J = 7.8 Hz, 2H), 7.41 (dd, J = 7.8, 4.8 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.17 – 7.11 (m, 1H), 7.11 – 7.05 (m, 1H), 5.14 (s, 2H), 4.27 (t, J = 5.5 Hz, 2H), 3.29 – 3.17 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.6, 157.5, 152.8, 150.7, 150.2, 135.9, 135.7, 134.2, 132.6, 132.2, 128.9, 125.5, 125.2, 125.0, 123.2, 121.7, 119.7, 117.4, 115.6, 110.8, 107.8, 47.7, 47.0, 23.4. HRMS (ESI): calcd for $C_{24}H_{20}N_5$ $[M+H]^+$, 378.1713; found, 378.1727.

2-(2-(Pyridin-3-yl)quinazolin-4-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (9i).: Yellow solid (yield: 62%). 1H NMR (500 MHz, $CDCl_3$) δ 9.75 (s, 1H), 8.79 (d, J = 7.9 Hz, 1H), 8.68 (d, J = 3.7 Hz, 1H), 8.38 (s, 1H), 8.02 – 7.91 (m, 2H), 7.73 (t, J = 7.6 Hz, 1H), 7.54 (d, J = 7.6 Hz, 1H), 7.48 – 7.42 (m, 1H), 7.39 (dd, J = 7.8, 4.8 Hz, 1H), 7.35 (d, J = 7.9 Hz, 1H), 7.21 – 7.10 (m, 2H), 4.99 (s, 2H), 4.14 (t, J = 5.3 Hz, 2H), 3.18 (d, J = 4.7 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.5, 157.4, 152.6, 150.7, 150.1, 136.3, 135.7, 134.1, 132.7, 130.9, 129.0, 127.0, 125.4, 124.6, 123.2, 121.8, 119.7, 118.0, 115.5, 111.0, 108.9, 49.8, 46.8, 22.0. HRMS (ESI): calcd for $C_{24}H_{20}N_5$ $[M+H]^+$, 378.1713; found, 378.1728.

4-(2-(Pyridin-3-yl)quinazolin-4-yl)-1,4-oxazepane (9j).: White solid (yield: 58%). 1H NMR (500 MHz, $CDCl_3$) δ 9.71 (d, J = 1.0 Hz, 1H), 8.81 – 8.57 (m, 2H), 7.93 (dd, J = 12.0, 8.4 Hz, 2H), 7.74 – 7.66 (m, 1H), 7.43 – 7.34 (m, 2H), 4.18 – 4.10 (m, 4H), 4.03 – 3.99 (m, 2H), 3.85 – 3.80 (m, 2H), 2.21 (p, J = 5.7 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.3, 157.2, 153.1, 150.6, 150.1, 135.5, 132.3, 128.9, 125.2, 124.5, 114.9, 70.2, 70.1, 53.7, 50.3, 30.7. HRMS (ESI): calcd for $C_{18}H_{19}N_4O$ $[M+H]^+$, 307.1553; found, 307.1557.

4-(2-(Pyridin-3-yl)quinazolin-4-yl)-2,3,4,5-tetrahydrobenzo[*ff*][1,4]oxazepine (9k): Yellow solid (yield: 55%). ¹H NMR (500 MHz, CDCl₃) δ 9.62 (s, 1H), 8.72 – 8.59 (m, 2H), 7.94 (t, *J* = 8.1 Hz, 2H), 7.77 – 7.68 (m, 1H), 7.45 – 7.39 (m, 1H), 7.39 – 7.31 (m, 2H), 7.22 (d, *J* = 7.5 Hz, 1H), 7.10 (t, *J* = 7.2 Hz, 1H), 7.02 (d, *J* = 7.9 Hz, 1H), 4.97 (s, 2H), 4.48 – 4.39 (m, 2H), 4.35 – 4.26 (m, 2H). ¹³C NMR (125 MHz, CDCl₃) δ 164.4, 159.3, 157.4, 152.8, 150.7, 150.2, 135.6, 132.6, 129.9, 129.0, 128.9, 128.7, 125.2, 125.1, 123.1, 120.5, 115.2, 70.9, 54.5, 54.0. HRMS (ESI): calcd for C₂₂H₁₉N₄O [M+H]⁺, 355.1553; found, 355.1565.

***N*-Methyl-*N*-(naphthalen-2-ylmethyl)-2-(pyridin-3-yl)quinazolin-4-amine (9l):** Off-white solid (yield: 52%). ¹H NMR (500 MHz, CDCl₃) δ 9.77 (s, 1H), 8.83 (d, *J* = 7.8 Hz, 1H), 8.69 (d, *J* = 3.5 Hz, 1H), 7.99 (d, *J* = 8.6 Hz, 2H), 7.95 – 7.78 (m, 4H), 7.78 – 7.67 (m, 1H), 7.58 – 7.44 (m, 3H), 7.41 (dd, *J* = 7.7, 4.9 Hz, 1H), 7.37 – 7.28 (m, 1H), 5.23 (s, 2H), 3.45 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 164.0, 157.5, 152.9, 150.8, 150.3, 135.8, 134.9, 134.3, 133.6, 133.0, 132.6, 129.0, 128.9, 127.9, 126.6, 126.1, 125.9, 125.3, 125.2, 125.1, 123.3, 115.1, 57.4, 39.7. HRMS (ESI): calcd for C₂₅H₂₁N₄ [M+H]⁺, 377.1761; found, 377.1775.

***N*-(Benzo[*c*][1,2,5]thiadiazol-5-ylmethyl)-*N*-methyl-2-(pyridin-3-yl)quinazolin-4-amine (9m):** Pale yellow solid (yield: 65%). ¹H NMR (500 MHz, CDCl₃) δ 9.70 (d, *J* = 1.6 Hz, 1H), 8.77 (dt, *J* = 7.9, 1.8 Hz, 1H), 8.67 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.07 – 8.02 (m, 2H), 8.00 (t, *J* = 8.1 Hz, 2H), 7.79 – 7.70 (m, 1H), 7.64 (dd, *J* = 9.1, 1.4 Hz, 1H), 7.43 – 7.32 (m, 2H), 5.24 (s, 2H), 3.49 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 163.9, 157.5, 155.2, 154.6, 153.1, 150.9, 150.3, 139.7, 135.7, 134.1, 132.8, 129.5, 129.2, 125.2, 125.0, 123.3, 122.2, 119.2, 115.1, 56.8, 40.3. HRMS (ESI): calcd for C₂₁H₁₇N₆S [M+H]⁺, 385.1230; found, 385.1245.

***N*-Methyl-*N*-((2-methylisoindolin-5-yl)methyl)-2-(pyridin-3-yl)quinazolin-4-amine (9n):** Light-brown solid (yield: 61%). ¹H NMR (400 MHz, CDCl₃) δ 9.73 (d, *J* = 1.5 Hz, 1H), 8.79 (dt, *J* = 8.0, 1.9 Hz, 1H), 8.68 (dd, *J* = 4.8, 1.7 Hz, 1H), 7.99 – 7.91 (m, 2H), 7.77 – 7.68 (m, 1H), 7.39 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.36 – 7.29 (m, 1H), 7.28 – 7.21 (m, 4H), 5.05 (s, 2H), 3.97 (d, *J* = 6.7 Hz, 4H), 3.38 (s, 3H), 2.64 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 163.9, 157.6, 153.0, 150.8, 150.3, 136.2, 135.7, 134.3, 132.5, 128.9, 126.1, 125.2, 125.0, 123.2, 122.8, 121.1, 115.1, 60.9, 60.8, 57.1, 42.4, 39.6. HRMS (ESI): calcd for C₂₄H₂₄N₅ [M+H]⁺, 382.2026; found, 382.2040.

***N*-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-*N*-methyl-2-(pyridin-3-yl)quinazolin-4-amine (9o):** Yellow solid (yield: 43%). ¹H NMR (400 MHz, CDCl₃) δ 9.74 (s, 1H), 8.80 (d, *J* = 8.0 Hz, 1H), 8.68 (d, *J* = 3.3 Hz, 1H), 7.97 (d, *J* = 8.8 Hz, 2H), 7.76 – 7.66 (m, 1H), 7.40 (dd, *J* = 7.8, 4.8 Hz, 1H), 7.37 – 7.32 (m, 1H), 6.89 (d, *J* = 9.4 Hz, 2H), 6.83 (d, *J* = 7.8 Hz, 1H), 5.97 (s, 2H), 4.98 (s, 2H), 3.37 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 163.8, 157.5, 152.9, 150.8, 150.3, 148.4, 147.2, 135.8, 132.5, 131.2, 128.9, 125.2, 125.0, 123.3, 120.7, 115.1, 108.6, 107.8, 101.2, 56.7, 39.6. HRMS (ESI): calcd for C₂₂H₁₉N₄O₂ [M+H]⁺, 371.1503; found, 371.1518.

***N*-((2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)methyl)-*N*-methyl-2-(pyridin-3-yl)quinazolin-4-amine (9p):** Pale-yellow solid (yield: 36%). ¹H NMR (500 MHz, CDCl₃) δ 9.73 (s, 1H), 8.79 (d, *J* = 7.9 Hz, 1H), 8.67 (d, *J* = 4.6 Hz, 1H), 7.95 (t, *J* = 8.0 Hz, 2H), 7.74 – 7.66 (m,

1H), 7.39 (dd, $J = 7.7, 4.9$ Hz, 1H), 7.36 – 7.30 (m, 1H), 6.94 (s, 1H), 6.89 (s, 2H), 4.95 (s, 2H), 4.27 (s, 4H), 3.37 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.8, 157.5, 153.0, 150.8, 150.3, 144.0, 143.1, 135.7, 134.3, 132.5, 130.6, 128.9, 125.2, 124.9, 123.2, 120.3, 117.7, 116.1, 115.1, 64.5, 64.4, 56.5, 39.5. HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{21}\text{N}_4\text{O}_2$ $[\text{M}+\text{H}]^+$, 385.1659; found, 385.1683.

(S)-N-((2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)methyl)-N-methyl-2-(pyridin-3-yl)quinazolin-4-amine (9q).: Pale-yellow solid (yield: 85%). ^1H NMR (500 MHz, CDCl_3) δ 9.65 (s, 1H), 8.72 (d, $J = 7.9$ Hz, 1H), 8.69 – 8.64 (m, 1H), 8.13 (d, $J = 8.4$ Hz, 1H), 7.93 (d, $J = 8.4$ Hz, 1H), 7.76 – 7.68 (m, 1H), 7.43 – 7.34 (m, 2H), 6.90 – 6.78 (m, 4H), 4.84 – 4.76 (m, 1H), 4.40 – 4.27 (m, 2H), 4.17 – 4.08 (m, 2H), 3.66 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.0, 157.1, 153.1, 150.8, 150.0, 143.1, 142.6, 135.5, 134.1, 132.5, 128.9, 125.5, 124.7, 123.2, 121.8, 121.5, 117.4, 117.2, 115.0, 71.8, 66.0, 53.0, 43.1. HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{21}\text{N}_4\text{O}_2$ $[\text{M}+\text{H}]^+$, 385.1659; found, 385.1657.

(R)-N-((2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)methyl)-N-methyl-2-(pyridin-3-yl)quinazolin-4-amine (9r).: Pale-yellow solid (yield: 89%). ^1H NMR (500 MHz, CDCl_3) δ 9.65 (s, 1H), 8.72 (d, $J = 7.9$ Hz, 1H), 8.69 – 8.64 (m, 1H), 8.13 (d, $J = 8.4$ Hz, 1H), 7.93 (d, $J = 8.4$ Hz, 1H), 7.76 – 7.68 (m, 1H), 7.43 – 7.34 (m, 2H), 6.90 – 6.78 (m, 4H), 4.84 – 4.76 (m, 1H), 4.40 – 4.27 (m, 2H), 4.17 – 4.08 (m, 2H), 3.66 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.0, 157.1, 153.1, 150.8, 150.0, 143.1, 142.6, 135.5, 134.1, 132.5, 128.9, 125.5, 124.7, 123.2, 121.8, 121.5, 117.4, 117.2, 115.0, 71.8, 66.0, 53.0, 43.1. HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{21}\text{N}_4\text{O}_2$ $[\text{M}+\text{H}]^+$, 385.1659; found, 385.1653.

N-Methyl-2-(pyridin-3-yl)-N-(quinoxalin-2-ylmethyl)quinazolin-4-amine (9s).: Yellow solid (yield: 47%). ^1H NMR (500 MHz, CDCl_3) δ 9.65 (s, 1H), 9.02 (s, 1H), 8.70 (d, $J = 7.7$ Hz, 1H), 8.65 (d, $J = 2.4$ Hz, 1H), 8.14 (d, $J = 8.4$ Hz, 1H), 8.10 (d, $J = 8.1$ Hz, 2H), 7.99 (d, $J = 8.3$ Hz, 1H), 7.83 – 7.72 (m, 3H), 7.42 (t, $J = 7.6$ Hz, 1H), 7.35 (dd, $J = 7.2, 5.0$ Hz, 1H), 5.41 (s, 2H), 3.63 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.5, 157.4, 153.2, 153.1, 150.8, 150.1, 144.7, 142.1, 135.7, 134.0, 132.8, 130.4, 129.9, 129.4, 129.2, 129.1, 125.3, 125.2, 123.3, 115.0, 57.0, 41.4. HRMS (ESI): calcd for $\text{C}_{23}\text{H}_{19}\text{N}_6$ $[\text{M}+\text{H}]^+$, 379.1666; found, 379.1682.

N-(Benzo[*d*]thiazol-2-ylmethyl)-N-methyl-2-(pyridin-3-yl)quinazolin-4-amine (9t).: Yellow solid (yield: 26%). ^1H NMR (400 MHz, CDCl_3) δ 9.80 (s, 1H), 8.84 (d, $J = 8.0$ Hz, 1H), 8.69 (d, $J = 3.6$ Hz, 1H), 8.13 (d, $J = 8.3$ Hz, 1H), 8.02 (dd, $J = 16.6, 8.2$ Hz, 2H), 7.81 (d, $J = 7.9$ Hz, 1H), 7.78 (d, $J = 8.1$ Hz, 1H), 7.48 (d, $J = 7.3$ Hz, 1H), 7.46 – 7.34 (m, 3H), 5.44 (s, 2H), 3.58 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 168.5, 163.2, 157.2, 153.1, 152.8, 150.9, 150.4, 135.9, 135.8, 133.8, 132.9, 129.1, 126.2, 125.4, 125.3, 125.3, 123.3, 123.0, 121.9, 115.0, 54.8, 40.8. HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{18}\text{N}_5\text{S}$ $[\text{M}+\text{H}]^+$, 384.1277; found, 384.1293.

N-((1H-Benzo[*d*]imidazol-2-yl)methyl)-N-methyl-2-(pyridin-3-yl)quinazolin-4-amine (9u).: Yellow solid (yield: 41%). ^1H NMR (400 MHz, CDCl_3) δ 9.61 (s, 1H), 8.66 (dt, $J = 8.0, 1.9$ Hz, 1H), 8.61 – 8.48 (m, 1H), 8.03 (d, $J = 8.3$ Hz, 1H), 7.92 (d, $J = 8.3$ Hz, 1H), 7.79 – 7.68 (m, 1H), 7.57 (brs, 2H), 7.46 – 7.33 (m, 1H), 7.26 – 7.20 (m, 3H), 5.27 (s, 2H), 3.47

(s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 163.4, 156.9, 152.9, 151.1, 150.7, 149.5, 135.5, 133.7, 133.2, 128.9, 125.6, 125.5, 123.4, 122.8, 114.9, 50.5, 40.7. HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{19}\text{N}_6$ $[\text{M}+\text{H}]^+$, 367.1666; found, 367.1683.

***N*-(Benzo[d]oxazol-2-ylmethyl)-*N*-methyl-2-(pyridin-3-yl)quinazolin-4-amine (9v).**: Off-white solid (yield: 35%). ^1H NMR (500 MHz, CDCl_3) δ 9.66 (d, J = 1.5 Hz, 1H), 8.73 (dt, J = 7.9, 1.8 Hz, 1H), 8.65 (dd, J = 4.8, 1.6 Hz, 1H), 8.21 (d, J = 7.9 Hz, 1H), 7.99 (d, J = 8.3 Hz, 1H), 7.82 – 7.75 (m, 1H), 7.75 – 7.70 (m, 1H), 7.59 – 7.51 (m, 1H), 7.50 – 7.44 (m, 1H), 7.38 – 7.30 (m, 3H), 5.25 (s, 2H), 3.71 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.5, 163.1, 157.2, 153.0, 150.9, 150.7, 150.1, 141.1, 135.9, 134.0, 132.9, 129.1, 125.4, 125.3, 125.3, 124.7, 123.3, 120.2, 115.1, 110.8, 50.4, 41.7. HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{18}\text{N}_5\text{O}$ $[\text{M}+\text{H}]^+$, 368.1506; found, 368.1518.

***N*-Methyl-*N*-(2-phenoxyethyl)-2-(pyridin-2-yl)quinazolin-4-amine (11a).**: Pale-yellow solid (yield: 73%). ^1H NMR (500 MHz, CDCl_3) δ 8.85 (d, J = 4.7 Hz, 1H), 8.48 (d, J = 7.9 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 8.13 (d, J = 8.5 Hz, 1H), 7.81 (td, J = 7.7, 1.8 Hz, 1H), 7.71 (ddd, J = 8.3, 7.0, 1.3 Hz, 1H), 7.40 (ddd, J = 8.3, 7.0, 1.2 Hz, 1H), 7.37 – 7.32 (m, 1H), 7.27 – 7.24 (m, 2H), 6.97 – 6.87 (m, 3H), 4.44 (t, J = 5.6 Hz, 2H), 4.28 (t, J = 5.6 Hz, 2H), 3.61 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.5, 158.5, 157.9, 156.0, 153.2, 150.0, 136.6, 132.2, 129.8, 129.5, 125.2, 124.9, 124.2, 123.6, 121.1, 115.3, 114.5, 65.8, 52.4, 41.7. HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{21}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 357.1710; found, 357.1724.

***N*-methyl-*N*-(2-phenoxyethyl)-2-(pyridin-4-yl)quinazolin-4-amine (11b).**: White solid (yield: 56%). ^1H NMR (500 MHz,) δ 9.69 (s, 1H), 8.75 (dd, J = 7.9, 1.7 Hz, 1H), 8.67 (d, J = 4.0 Hz, 1H), 8.13 (d, J = 8.5 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.73 – 7.68 (m, 1H), 7.41 – 7.35 (m, 2H), 7.26 (d, J = 8.4 Hz, 2H), 6.95 – 6.87 (m, 3H), 4.45 (t, J = 5.4 Hz, 2H), 4.28 (t, J = 5.4 Hz, 2H), 3.68 (s, 3H), 3.63 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.2, 158.5, 157.0, 152.9, 150.2, 146.2, 132.4, 129.6, 129.0, 125.5, 125.0, 122.2, 121.1, 115.3, 114.5, 65.8, 52.5, 41.7. HRMS (ESI): calcd for $\text{C}_{22}\text{H}_{21}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$, 357.1710; found, 357.1721.

***N*-Methyl-*N*-(2-phenoxyethyl)-2-(pyrimidin-5-yl)quinazolin-4-amine (11c).**: White solid (yield: 61%). ^1H NMR (500 MHz, CDCl_3) δ 9.73 (s, 2H), 9.27 (s, 1H), 8.15 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 8.3 Hz, 1H), 7.76 – 7.70 (m, 1H), 7.45 – 7.40 (m, 1H), 7.29 – 7.24 (m, 2H), 6.93 (t, J = 7.4 Hz, 1H), 6.89 (d, J = 7.9 Hz, 2H), 4.44 (t, J = 5.3 Hz, 2H), 4.28 (t, J = 5.3 Hz, 2H), 3.64 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 162.0, 158.2, 157.4, 155.7, 154.0, 151.8, 131.5, 130.8, 128.5, 127.8, 124.6, 124.0, 120.1, 114.2, 113.4, 64.8, 51.5, 40.9. HRMS (ESI): calcd for $\text{C}_{21}\text{H}_{20}\text{N}_5\text{O}$ $[\text{M}+\text{H}]^+$, 358.1662; found, 358.1673.

***N*-Methyl-*N*-(2-phenoxyethyl)-2-phenylquinazolin-4-amine (11d).**: White solid (yield: 82%). ^1H NMR (500 MHz, CDCl_3) δ 8.54 – 8.47 (m, 2H), 8.11 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 8.2 Hz, 1H), 7.69 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 7.48 – 7.43 (m, 3H), 7.36 (ddd, J = 8.3, 6.9, 1.2 Hz, 1H), 7.28 – 7.24 (m, 2H), 6.97 – 6.89 (m, 3H), 4.45 (t, J = 5.6 Hz, 2H), 4.27 (t, J = 5.6 Hz, 2H), 3.60 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 163.2, 159.0, 158.6, 132.1, 130.0, 129.5, 128.4, 128.3, 125.4, 124.2, 121.0, 114.5, 65.9, 52.4, 41.8. HRMS (ESI): calcd for $\text{C}_{21}\text{H}_{20}\text{N}_6$ $[\text{M}+\text{H}]^+$, 356.1744; found, 356.1771.

N-Methyl-N-(2-phenoxyethyl)-2-(thiophen-3-yl)quinazolin-4-amine (11e).: Pale-yellow solid (yield: 71%). ¹H NMR (500 MHz, CDCl₃) δ 8.26 (s, 1H), 8.08 (d, *J* = 8.4 Hz, 1H), 7.94 (dd, *J* = 5.0, 0.9 Hz, 1H), 7.88 (d, *J* = 7.5 Hz, 1H), 7.69 – 7.63 (m, 1H), 7.37 – 7.30 (m, 2H), 7.26 (dd, *J* = 8.6, 7.5 Hz, 2H), 6.96 – 6.90 (m, 3H), 4.43 (t, *J* = 5.6 Hz, 2H), 4.23 (t, *J* = 5.6 Hz, 2H), 3.58 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 163.2, 158.6, 156.4, 153.1, 142.8, 132.1, 129.5, 128.5, 127.8, 127.2, 125.4, 124.0, 121.0, 114.8, 114.6, 65.9, 52.4, 41.7. HRMS (ESI): calcd for C₂₁H₂₀N₃OS [M+H]⁺, 362.1322; found, 362.1336.

N-Methyl-N-(2-phenoxyethyl)-2-(thiophen-2-yl)quinazolin-4-amine (11f).: Pale-yellow solid (yield: 45%). ¹H NMR (500 MHz, CDCl₃) δ 8.06 (d, *J* = 8.4 Hz, 1H), 8.00 (s, 1H), 7.86 (s, 1H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.41 (d, *J* = 4.8 Hz, 1H), 7.36 – 7.29 (m, 1H), 7.28 – 7.24 (m, 2H), 7.14 – 7.11 (m, 1H), 6.95 – 6.89 (m, 3H), 4.46 (t, *J* = 5.5 Hz, 2H), 4.22 (t, *J* = 5.5 Hz, 2H), 3.60 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 162.8, 158.6, 155.9, 153.0, 145.0, 132.3, 129.5, 128.8, 128.3, 128.0, 125.5, 124.0, 121.0, 114.8, 114.5, 65.9, 52.6, 41.9. HRMS (ESI): calcd for C₂₁H₂₀N₃OS [M+H]⁺, 362.1322; found, 362.1336.

2-(Furan-2-yl)-N-methyl-N-(2-phenoxyethyl)quinazolin-4-amine (11g).: Yellow oil (yield: 40%). ¹H NMR (500 MHz, CDCl₃) δ 8.06 (dd, *J* = 8.5, 0.9 Hz, 1H), 7.95 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.67 (ddd, *J* = 8.3, 6.9, 1.3 Hz, 1H), 7.61 (dd, *J* = 1.6, 0.8 Hz, 1H), 7.34 (ddd, *J* = 8.3, 6.9, 1.3 Hz, 1H), 7.29 – 7.22 (m, 3H), 6.96 – 6.90 (m, 3H), 6.53 (dd, *J* = 3.3, 1.7 Hz, 1H), 4.42 (t, *J* = 5.6 Hz, 2H), 4.20 (t, *J* = 5.6 Hz, 2H), 3.56 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 163.2, 158.5, 153.2, 152.8, 152.5, 144.5, 132.3, 129.5, 128.7, 125.4, 124.2, 121.0, 115.0, 114.5, 112.7, 111.7, 65.7, 52.4, 41.7. HRMS (ESI): calcd for C₂₁H₂₀N₃O₂ [M+H]⁺, 346.1550; found, 346.1559.

2-(Furan-3-yl)-N-methyl-N-(2-phenoxyethyl)quinazolin-4-amine (11h).: Yellow solid (yield: 63%). ¹H NMR (500 MHz, CDCl₃) δ 8.21 (s, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 7.85 (d, *J* = 8.3 Hz, 1H), 7.69 – 7.63 (m, 1H), 7.47 (t, *J* = 1.7 Hz, 1H), 7.35 – 7.29 (m, 1H), 7.29 – 7.24 (m, 2H), 7.11 (d, *J* = 1.5 Hz, 1H), 6.96 – 6.92 (m, 1H), 6.91 (d, *J* = 7.9 Hz, 2H), 4.41 (t, *J* = 5.6 Hz, 2H), 4.19 (t, *J* = 5.6 Hz, 2H), 3.55 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 163.1, 158.6, 155.7, 153.0, 144.5, 143.4, 132.1, 129.5, 128.3, 127.9, 125.4, 123.9, 121.0, 114.9, 114.5, 109.9, 65.8, 52.3, 41.6. HRMS (ESI): calcd for C₂₁H₂₀N₃O₂ [M+H]⁺, 346.1550; found, 346.1561.

Enzyme Activity Assay—4-Methylumbelliferyl β -D-glucopyranoside (4MU- β -Glc) and buffer components were purchased from Sigma-Aldrich (St. Louis, MO). Residual injection solutions of the recombinant wild-type GCcase enzyme velaglucerase alfa (Vpriv[®], Shire Human Genetic Therapies, Inc.) in clinics were collected for the activity assays. The GCcase activity assay buffer was composed of 50 mM citric acid, 176 mM K₂HPO₄, and 0.01% Tween-20 at pH 5.9. A solution of 1 M sodium hydroxide and 1 M glycine was used as the stop solution.

The compounds in DMSO solution (0.5 μL/well) were transferred to a black 96-well plate (the final titration was 48 nM to 100 μM, a 12-point dilution series). The enzyme solution (33.5 μL, 7.5 nM final concentration) was transferred to the wells. After 5 min of incubation at room temperature, the enzyme reaction was initiated by the addition of blue substrate

(4MU- β -Glc) (33 μ L/well). The final concentration of the blue substrate was 1.5 mM. The blue substrate reaction was terminated by the addition of 33 μ L/well stop solution (1 M NaOH and 1 M glycine mixture, pH 10) after 30 min of incubation at 37 °C. The fluorescence was then measured in a Biotek Synergy H1 multi-mode plate reader with λ_{ex} = 365 nm and λ_{em} = 440 nm.

Cell Culture and Compound Treatment—The GBA N370S/84GG fibroblast cell line was obtained from Coriell, GM00372, cultured in DMEM medium (Life Tech) including 1% v/v L-glutamine 200 mM (Life Tech), 1% v/v pen strep (Life Tech), 10% FBS (Life Tech) at 37 °C and 5% CO₂ and treated with different compounds at indicated concentrations. The same volume of DMSO (0.1% v/v) was used as a control. After a 3-day treatment for fibroblasts or a 10-day treatment for dopaminergic neurons, cells were washed with PBS twice followed by 1% Triton X-100 lysis buffer to lyse cells. Protein concentrations were measured with a BCA kit (Thermo), and the GCase activity was determined at pH 5.5.

Measurement of Lysosomal GCase Activity—Fibroblasts were plated into a 96-well plate and cultured and treated with compound as described above. After 3 days of treatment, the medium was replaced with media containing 5-(pentafluorobenzoylamino)fluorescein di- β -D-glucopyranoside (PFB-FDGlu) or media containing PFB-FDGlu with 400 μ M bafilomycin. Cells were incubated for 1 h to allow for loading of the substrate into the lysosome. After loading, substrate hydrolysis was monitored by measuring fluorescence unquenching every 20 min at 485 nm excitation and 525 nm emission.

Human iPSC Culture and Neural Differentiation—Human iPSC lines were generated from skin fibroblasts through retroviral expression or using Sendai Virus based delivery of key reprogramming vectors including four Yamanaka factors (Oct, Sox2, Klf4 and c-Myc)²³. Heterozygous N370S *GBA1* mutant fibroblasts were reprogrammed into iPSC through Northwestern University Stem Cell Core Facility and characterized in this study (see Supporting Information figure).

The iPSC line from one separate healthy control subject was previously described and characterized²⁴. Healthy control and *GBA1* mutant iPSC lines were characterized for expression of pluripotent markers by immunocytochemistry (NANOG, OCT4, SSEA4, TRA1–81). Karyotype analysis was performed by Cell Line Genetics using standard protocols for high-resolution G-banding. iPSC were cultured in mTeSR media (Stem Cell Technologies) and passaged every 5 to 7 days on matrigel (Corning) coated plates. All lines were routinely tested for mycoplasma contamination.

Differentiation of iPSCs into midbrain dopaminergic neurons was done according to published protocols²⁵, passaged and maintained as described previously²⁶. At d25 to d30, neural blocks were passed by accutase treatment onto PDL / laminin coated culture dishes. Neuralization growth factors were withdrawn at d40 and neurons were maintained in Neurobasal media (Life Technologies) containing Neurocult SM1 (Stemcell technologies). Immunocytochemistry demonstrated the neuralization efficiency in neurons derived from iPSC lines using neural (β -III-tubulin) and midbrain (TH, FOXA2, LMX1a) specific markers.

Sequencing Analysis—DNA samples were extracted from cell pellets using Mini prep kits (Qiagen). Eluted gDNA was used as a template for PCR amplification using Phusion Master Mix (NEB). PCR product was purified using PCR Purification Kit (Qiagen) and sent for sequencing through Northwestern University Seq Core or University of Chicago Seq Core.

Deglycosylation of Proteins/Molecular Shift Assay²²—To study the subcellular localization and transport of the various GC mutants (ER and post-ER localization), EndoH and PNGaseF digestions were performed. For both reactions 20 µg of protein was used, and the experimental procedure was performed according to the manufacturer's handbook (New England Biolabs). A positive digestion resulted in a shift in molecular size after the protein was subjected to SDS/PAGE. Anti-hGC (From Johannes Aerts, Leiden University, Leiden, The Netherlands) was used to detect the different forms of GC.

Western Blots—Proteins were denatured in 20% SDS sample buffer at 100 °C for 10 min; 10% Tris-glycine (Life Tech) was used for gels; a Trans-Blot Turbo PVDF kit (Bio-Rad) was used for membrane transfer. GCase antibody (Sigma-Aldrich) and GAPDH primary antibodies (EMD Millipore) were incubated with the membranes overnight, then the incubated membranes were treated with the secondary antibody (IRDye 680RD Goat anti-Rabbit IgG (H+L) or IRDye 800CW Goat anti-Mouse IgG (H+L), LI-COR) for 30 min. An Odyssey CLx Imaging System (LI-COR) was used to scan the membranes and analyze the imaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

GD	Gaucher's disease
GCase	β-glucocerebrosidase
ER	endoplasmic reticulum
PC	pharmacological chaperone
IFG	Isofagomine
PD	Parkinson's Disease

DLB dementia with Lewy bodies
SAR structure activity relationship

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Figure 1.
Structures of GCase inhibitors and activators

A. **B.** **Figure 2.**

GCCase enzyme activity switch by *N*-methylation of quinazoline derivatives. (A) Example of the change in activity by *N*-methylation; (B) Plot demonstrating the change in activity from inhibition (**5a**) to activation (**6a**)

Figure 3.
Modification of the *N*-substituent by oxygen replacement (**7a**) and *N*-methylation (**8a**)

Figure 4.

Activator **9q** increases GCase protein levels in (A) healthy control fibroblasts and (B) compound heterozygous *GBA1* mutant fibroblasts (N370S/84GG) derived from a GD patient. Cell lysates from fibroblasts treated with vehicle (DMSO), **9q** (5 μ M, 15 μ M), or isofagomine (IFG) were analyzed by immunoblotting after no digestion (top), Endo H digestion (middle), and PNGase F digestion (bottom); n = 4.

Figure 5.

GCase enzyme activity in cell lysates and in the lysosome of **9q**-treated cells. GCase activity was measured in lysates from (A) healthy control fibroblasts and (B) compound heterozygous *GBA1* mutant fibroblasts (N370S/84GG). Lysosomal GCase was measured using a live-cell assay in (C) healthy control, (D) homozygous N370S, and (E) homozygous L444P mutant fibroblasts after 3-day treatment with **9q** (5 μ M, 15 μ M), 5 μ M isofagomine (IFG), or vehicle (DMSO). The data are presented as the mean $\pm$ SEM, $n = 3-4$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus vehicle treatments; one-way ANOVA was followed by the Tukey's multiple comparisons *post hoc* test.

Figure 6.

Activator **9q** increases GCase protein levels and enzyme activity in (A) wild-type control and (B) patient-derived heterozygous N370S *GBA1* mutant dopaminergic neurons at day 70 of differentiation. Cell lysates from neurons treated with vehicle (DMSO) or **9q** (5 μ M, 15 μ M) for 10 consecutive days were analyzed by immunoblotting after no digestion (top), Endo H digestion (middle), and PNGase F digestion (bottom); n = 3.

Fig 7. GCase enzyme activity in protein lysates of (A) wild-type control and (B) patient-derived heterozygous N370S *GBA1* mutant dopaminergic neurons after 10 days consecutive treatment with vehicle (DMSO) or **9q** (day 70 of differentiation). The data are presented as the mean $\pm$ SEM, $n = 4-7$; * $p < 0.05$, ** $p < 0.01$ versus vehicle treatments; one-way ANOVA was followed by the Tukey's multiple comparisons *post hoc* test.

Reagents and conditions: (a) (i) sulfolane (ii) PCl_5 ; 46%; (b) R^1NH_2 , K_2CO_3 , DMF; 34-73%; (c) (i) NaH, DMF (ii) R^2I ; (d) $\text{R}^1\text{R}^2\text{NH}$, K_2CO_3 , DMF; 33-96%; (e) RH , K_2CO_3 , DMF; 26-89%.

Scheme 1.
Synthesis of *N*-methyl quinazoline derivatives

Reagents and conditions: (a) *N*-methyl-2-phenoxyethanamine, K_2CO_3 , DMF, 80%;

(b) $ArB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3 , 1,4-dioxane, H_2O ; 40-82%; (c)

N-methyl-2-phenoxyethanamine, K_2CO_3 , DMF, 73%;

Scheme 2.

Synthesis of **11a-11i** with modifications at the 2-position of the quinazoline ring

Table 1.Structure and enzyme activity of quinazoline derivatives without and with the *N*-methyl substituent^a

Cmpd	R ¹	R ²	IC ₅₀ (μM)	AC ₅₀ (μM)	E _{max} (%)
5a		H	1.78 ± 0.22		
6a		CH ₃		5.01 ± 0.24	223
5b		H	0.097 ± 0.009		
6b		CH ₃		1.39 ± 0.26	126
5c		H	1.28 ± 0.13		
6c		CH ₃		3.99 ± 0.17	190
5d		H	0.72 ± 0.05		
6d		CH ₃		6.36 ± 0.36	149
5e		H	0.177 ± 0.012		
6e		CH ₃		2.36 ± 0.69	137
5f		H	0.042 ± 0.003		
6f		CH ₃		9.94 ± 0.38	
5g		H	0.027 ± 0.002		
6g		CH ₃		8.38 ± 0.70

Cmpd	R ¹	R ²	IC ₅₀ (μM)	AC ₅₀ (μM)	E _{max} (%)
5h		H	0.0083 ± 0.001		
6h		CH ₃	2.14 ± 0.24		
5i		H	0.0087 ± 0.0011		
6i		CH ₃	0.43 ± 0.02		
5j		H	0.0099 ± 0.0013		
6j		CH ₃	5.87 ± 0.24		

^aExperiments were performed in triplicate, and the mean ± SD is shown.

Table 2.

Structures and inhibition or activation activity of quinazoline derivatives having an oxygen isostere and methylation in the *N*-substituent ^a

Cmpd	R ¹	R ²	IC ₅₀ (μM)	AC ₅₀ (μM)	E _{max} (%)
7a		H		1.56 ± 0.30	140
8a		CH ₃		2.30 ± 0.23	552
7b		H	7.30 ± 0.33		
8b		CH ₃		16.87 ± 0.27	394
7c		H	2.54 ± 0.12		
8c		CH ₃		6.16 ± 0.15	208
7d		H	4.24 ± 0.25		
8d		CH ₃		8.97 ± 0.41	390
7e		H	1.88 ± 0.21		
8e		CH ₃		7.52 ± 0.42	345
8f		CH ₂ CH ₃		5.38 ± 0.34	478
8g		CH ₂ CH ₂ CH ₃		3.18 ± 0.69	132
8h				Inactive	

^aExperiments were performed in triplicate, and the mean ± SD is shown

Table 3.

Structure and activation activity of quinazoline derivatives with conformationally-restricted substituents and fused aromatic ring substituents^a

Cmpd	R	AC ₅₀ (μM)	E _{max} (%)	Cmpd	R	AC ₅₀ (μM)	E _{max} (%)
9a		1.17 ± 0.09	276	9l		3.80 ± 0.03	155
9b		4.75 ± 0.09	237	9m		1.84 ± 0.08	337
9c		3.65 ± 0.34	302	9n		14.91 ± 0.95	428
9d		4.47 ± 0.37	364	9o		1.63 ± 0.13	160
9e		5.18 ± 0.08	160	9p		4.88 ± 0.29	488
9f		4.66 ± 0.22	130	9q		1.49 ± 0.06	780

Cmpd	R	AC ₅₀ (μM)	E _{max} (%)	Cmpd	R	AC ₅₀ (μM)	E _{max} (%)
9g		2.23 ± 0.13 ^b		9r		2.37 ± 0.14	296
9h		Inactive		9s		1.63 ± 0.02	674
9i		4.27 ± 0.17	175	9t		6.90 ± 0.94	517
9j		44.14 ± 4.49	423	9u		7.92 ± 1.64	197

Cmpd	R	AC ₅₀ (μM)	E _{max} (%)	Cmpd	R	AC ₅₀ (μM)	E _{max} (%)
9k		9.74 ± 1.10	438	9v		16.07 ± 0.56	643

^aExperiments were performed in triplicate, and the mean ± SD is shown

^bInhibitor

Table 4.

Structure and activation activity of *N*-methyl-*N*-(2-phenoxyethyl) quinazolin-4-amine derivatives containing aromatic rings (**11a-11g**)^a

Cmpd	R	AC ₅₀ (μM)	E _{max} (%)
8a		2.30 ± 0.23	552
11a		> 50	
11b		2.89 ± 0.34	690
11c		0.89 ± 0.01	634
11d		Inactive	
11e		Inactive

Cmpd	R	AC ₅₀ (μM)	E _{max} (%)
11f		Inactive	
11g		21.73 ± 7.67	229
11h		1.68 ± 0.09	145

^aExperiments were performed in triplicate, and the mean ± SD is shown